Proc. Nat. Acad. Sci. USA
Vol. 70, No. 4, pp. 969–972, April 1973

"Recommended Daily Allowances" for Vitamin C

(ascorbic acid/guinea pig/human nutrition/scurvy)

MAN-LI S. YEW

Clayton Foundation Biochemical Institute, University of Texas at Austin, Austin, Tex. 78712

Communicated by Roger J. Williams, January 29, 1973

ABSTRACT **Although 41 years have elapsed since the identification of vitamin C, the amounts required for human health are far from established. Pauling's suggestion that human needs for this vitamin probably have been underestimated by a factor of 10 or more has frequently been ignored or refuted by rhetoric rather than by sound experimentation.**

In the present study young healthy guinea pigs were fed a scorbutic diet supplemented with ascorbic acid at four widely different levels (0.05, 0.5, 5.0, and 50 mg/100 g of body weight per day). Growth rates both before and after surgical stress, recovery times after anesthesia, scab formation, wound healing, and the production of hydroxyproline and hydroxylysine during wound healing all support the conclusion that young guinea pigs ordinarily need about 5.0 mg/100 g of body weight daily. This is far beyond what is needed to prevent scurvy. Under stress the needs are even higher. On a body-weight basis this amount is equivalent to a need on the part of a 30-kg human child of 1500 mg of ascorbic acid daily. While calculations on a body-weight basis are subject to some uncertainty, the enormous discrepancy (nearly 40-fold) between this amount and that recommended by the Food and Nutrition Board calls attention to the extreme uncertainty about human ascorbic-acid needs, and to an important public health problem related to the best development of young people.

41 Years have now elapsed since vitamin C, ascorbic acid, was identified (1), and it seems that by now medical science should have definite information about the crucial problem of how much of this vitamin human beings—including particularly children—need to promote good health and development. This information would be available by now if consistent scientific effort had been expended to obtain it.

Due, however, to decades of neglect on the part of medical scientists, much practical information about human needs for various nutrients is shrouded in confusion and uncertainty.

Pauling (2) and Stone (3) are leaders in thinking that the human needs for ascorbic acid have been underestimated by a factor of 10 or more. Those who ignore this suggestion or have merely used rhetoric to refute it appear to accept axiomatically two crippling but time-honored *assumptions*, first, that ascorbic acid functions merely to prevent "scurvy," and second, that within a species a narrow range of needs must *of necessity* exist.

These assumptions need to be discarded in favor of objective and realistic investigation to ascertain the actual facts, in contrast to those which fit most gracefully into preconceived ideas and dogmas.

One important way of gaining information about the needs of human beings is to determine the requirements of other species, particularly guinea pigs—animals that are relatively convenient and inexpensive to study. The growing appreciation of the unity in nature makes this approach most useful.

There is at present great uncertainty with respect to guinea pig needs for ascorbic acid, as well as those of human beings. Williams and Deason (4) have presented direct evidence based, however, on only one response—growth rates—that guinea pig requirements vary greatly from animal to animal, and may be very high. Guinea pig requirements demand further study. It is difficult to imagine that human requirements will be clarified while guinea pig needs are so uncertain.

The present study has two advantages over many others: (*i*) it encompasses a very wide (1000-fold) range in ascorbic-acid intake levels, thus eliminating one undesirable assumption, and (*ii*) it involves the use of several criteria on which to base judgments of "adequacy," thus eliminating the assumption that deficiency, by definition, leads to "scurvy."

MATERIALS AND METHODS

50 Young male guinea pigs purchased from a commercial breeder were given Purina guinea pig chow until they reached an average weight of 350 g. Then they were evenly divided into five groups. One group was continued on the chow throughout the experiment. The other four groups were placed on an ascorbate-free diet* supplemented with ascorbic acid three times a week, so that there were four different levels of intake—i.e., 0.05, 0.5, 5.0, and 50.0 mg/100 g of body weight per day. The ascorbic acid was dissolved in 10% sucrose solution and was given orally with a medicine dropper. Food and water were available to all guinea pigs (individually caged) at all times except as indicated. Weights, as well as signs of illness, were carefully recorded at least three times a week. At the end of 30 days, all guinea pigs were shaved on the lower back and fasted overnight. The next morning, each animal was given intraperitoneally 0.1 ml of atropine sulfate, and 30 min later, sodium pentathal, at 3.2 mg/100 g of body weight. A circular piece of skin, 5 mm in diameter, was punched out on the back using a sharp paper puncture device. Each animal was returned to its cage with food and water and observed until it regained activity, and then observed daily for wound contraction and falling of scab. About 18 days later, the animals were again fasted overnight. A second skin sample was excised under the same anesthesia from the previous wound location (the regenerated skin tissue). The skin samples

* Modified Reid–Brigg's guinea pig test diet, modified to exclude ascorbic acid and to elevate levels of menadione to 0.045 gram/kg; α-tocopherol acetate to 100 mg/kg, and calciferol D_2 to 0.75 mg/kg.

TABLE 1. *Recovery time, wound healing, and growth rates before and after surgery at different levels of ascorbic-acid intake*

Level	Ascorbic acid per 100 g of body weight per day	Recovery from anesthesia, hr (ranges)	Falling of scabs, in days (ranges)	Average weight gain per day (ranges) (before surgery)	Average weight gain per day (ranges) (after surgery)
I	0.05	10.0*	18.5*	0.38	−3.34*
		(8-12)	(12-18+)	(−4.29-6.36)	(−4.85-0.21)
II	0.5	6.0	10.2	7.04	6.18
		(5.5-6.3)	(9-11)	(0.64-11.43)	(−4.35-9.00)
III	5.0	5.3	9.78	8.54	7.61
		(4.5-6.0)	(7-12)	(5.50-11.72)	(4.85-9.78)
IV	50.0	4.9	9.50	6.92	7.07
		(4.5-5.5)	(7-11)	(2.5-11.64)	(5.35-9.64)
	Purina guinea pig chow†	4.7	11.8	9.78	8.25
		(4.5-5.2)	(7-16)	(8.36-11.43)	(5.71-11.14)

* This is an average of six animals; 4 guinea pigs at this level of intake were removed from the experiment since they were judged to be in no condition to withstand surgery. Of the other 40 animals on other levels, only four were lost in surgery or accident.

† The Purina guinea pig chow contains 0.33 mg of ascorbic acid per g of diet at the time of feeding. Considering a growing guinea pig of about 500 g eating 50 g of the diet per day, its ascorbic acid intake would be in the vicinity of 3.3 mg/100 g of body weight per day. 18+, more than 18 days.

were cleaned of hairs, washed with ether, dehydrated, and hydrolyzed in 6 N HCl at 106° for 20 hr. Aminoacid analysis was done with a Beckman/Spinco aminoacid analyzer.

RESULTS

The major results of this study are summarized in Tables 1, 2, and 3.

DISCUSSION

Recovery from anesthesia

As shown in Tables 1 and 3, the recovery time from anesthesia of animals receiving ascorbic acid at level II (0.5 mg/100 g of body weight per day) was statistically shorter than those of the animals receiving level I (0.05 mg/100 g of body weight per day). Level III also showed a statistical advantage over level II, indicating that under these stressful conditions, using this criterion, guinea pigs require about 5 5 mg/100 g of body weight per day for best performance. Even animals at level IV seemed to have a slight advantage, though it was not statistically significant at the conventional 5% level. So far as we know, this criterion (recovery time from anesthesia) has never been used before to help assess the ascorbic-acid needs of guinea pigs.

Wound healing, release of scab

Guinea pigs receiving the lowest level of ascorbic acid (0.05 mg/100 g of body weight per day) exhibited very slow wound healing in comparison with the other groups (Tables 1 and 3). 2 of the 6 guinea pigs operated on receiving this level showed no scab formation for 18 days, and the other 4 showed

TABLE 2. *Aminoacid analysis of regenerated skin samples at different levels of ascorbic-acid intake (μmol of amino acid per 100-mg skin sample)*

Amino acid	Level I	Level II	Level III	Level IV	Control*
Hydroxyproline	Trace	24.87	37.95	35.03	95.55
Proline	42.23	44.98	65.64	62.78	129.91
Hydroxylysine	Trace	2.37	2.96	2.81	5.23
Lysine	38.06	38.35	34.67	34.59	29.67
Glycine	65.43	130.05	172.58	165.61	375.82
Aspartic acid	60.14	53.35	57.88	58.85	51.27
Threonine	39.49	27.92	30.12	30.04	20.76
Serine	40.85	44.65	47.94	46.00	38.74
Alanine	55.54	55.87	71.42	69.05	110.91
Valine	39.19	30.78	32.79	33.19	23.56
Leucine	51.66	42.91	44.37	48.34	26.27
Isoleucine	21.92	18.47	20.34	21.76	11.37
Tyrosine	15.96	15.20	14.44	14.62	5.75
Methionine	4.66	7.50	7.19	7.04	5.25
Phenylalanine	23.67	18.76	19.27	19.11	13.84
Histidine	19.70	11.59	9.87	11.57	5.28
Arginine	25.14	42.47	42.37	39.28	40.75
Glutamic acid	56.45	83.17	86.03	83.89	78.72

* Values are averages of amino acids of normal skin samples from guinea pigs maintained on Purina guinea pig chow.

TABLE 3. *Statistical differences observed at different levels of ascorbic-acid intake (t-tests)*

	Differences between		
	Levels I and II	Levels II and III	Levels III and IV
Recovery time	$P < 0.005$	$P < 0.005$	$0.1 < P < 0.15$
Scab release	$P < 0.005$	$P > 0.15$	$P > 0.15$
Growth rate (before surgery)	$P < 0.005$	$P \cong 0.15$	$P > 0.15$
Growth rate (after surgery)	$P < 0.005$	$P \cong 0.15$	$P > 0.15$
Aminoacid levels in regenerated skin			
Hydroxyproline	$P < 0.005$	$P < 0.005$	$P > 0.15$
Hydroxylysine	$P < 0.005$	$0.05 < P < 0.1$	$P > 0.15$
Proline	$P > 0.15$	$P < 0.005$	$P > 0.15$
Lysine	$P > 0.15$	$0.1 < P < 0.5$	$P > 0.15$
Glycine	$P < 0.005$	$P \cong 0.025$	$P > 0.15$
Alanine	$P > 0.15$	$P < 0.005$	$0.1 < P < 0.15$

much slower healing than those at higher levels. At the lowest level the regenerated tissue was pinkish and had a mushy texture, while at the higher levels of intake the tissues were whitish and of a collagenous texture.

Observation of wound healing, scab formation, and release did not yield any specific evidence that the need of guinea pigs for ascorbic acid is higher than 5 mg/100 g of body weight per day. Much higher levels, however, seemed to have no deleterious effects.

Growth rates

During the period before surgery, animals at level II had higher growth rates than those in level I ($P < 0.005$). Those at level III exhibited higher rates than those at level II and level IV, but not significantly so ($P \cong 0.15$ and $P > 0.15$). When growth rates before surgery are used as the criterion, guinea pigs requirements would be estimated in excess of 0.5 mg/100 g of body weight per day.

During the 18-day period after surgery the comparisons were as indicated in Tables 1 and 3. During this period the animals tended to gain much less, especially at the lower levels. Using weight gain after surgery as the criterion, we would have to conclude that the guinea pig requirement is about 5.0 mg/100 g of body weight per day. The highest level showed no statistical advantage, and possibly was too high for some of the animals.

Amino acids in regenerated skin

Analysis of the aminoacid content of regenerated skin obtained 18 days after the first surgery gave strong evidence that collagen building and aminoacid synthesis was greatly influenced by the ascorbic acid intake (Table 2).

As shown in Table 3, level II very significantly promoted the presence of higher levels of hydroxyproline, hydroxylysine, and glycine compared with level I. Level III significantly promoted the presence of higher levels of hydroxyproline, proline, glycine, and alanine, compared with level II.

These findings, which suggest further exploration, indicate that in guinea pigs after surgery the needs for ascorbic acid are apparently in the range of 5.0 mg/100 g of body weight per day. The evidence does not contraindicate that the needs may be higher than level III. Level IV appears to be higher than desirable.

Individuality

Individuality in guinea pig requirements for ascorbic acid is reflected throughout the study. One animal at the lowest level of intake, for example, exhibited good growth rates of 6.3 g/day, while another animal in the same group lost 4.2 g/day.

It is very noteworthy that the variation was greater at the lower levels of intake and was less at higher levels. This was most strikingly true with respect to growth rates both before and after surgery. The conclusion seems inevitable that the individual needs of guinea pigs vary greatly, but that when they are given relatively high levels of ascorbic acid, the whole population appears to be adequately supplied, with the result that the performance is more nearly uniform.

That intake levels may be too high for individual guinea pigs is suggested by the fact that one guinea pig receiving 50 mg/100 g of body weight per day (Table 1) gained only 2.5 grams/day. *Under stress* (after surgery), this same guinea pig gained 6.2 g/day.

Bearing of results on human nutrition

Our experiments, covering a 1000-fold range in ascorbic-acid consumption levels, were not designed to arrive at an exact requirement. It does seem clear, however, that young guinea pigs need *about* 5.0 mg/100 g of body weight per day for good health and development. This is a very high figure, and the question of whether it may be high or low by 1–2 mg does not concern us. We are more concerned about the order of magnitude.

One basis for accepting our approximate figure (5.0 mg/100 g of body weight per day) is the fact that the commercial guinea pig chow we used (which is obviously tailored realistically to meet the needs of guinea pigs) contains enough so that if they eat "normally", the animals get about 3.3 mg/100 g of body weight per day.

If, as our results show, (*i*) the requirement of young guinea pigs for ascorbic acid is very much higher than has commonly been supposed, (*ii*) ascorbic acid is not merely necessary for young guinea pigs to protect against guinea pig scurvy, and (*iii*) the needs of individual guinea pigs vary over a wide range, then it follows that *most probably* (*i*) the requirement of young human beings for ascorbic acid is very much higher than has been supposed, (*ii*) ascorbic acid is not merely needed by young people to prevent human scurvy, and (*iii*) the individual needs of young people for ascorbic acid vary over a wide range.

The burden of proof must now shift to the medical scientists who are really concerned about the health and physical condition of young people to show that considerations that apply to young guinea pigs do not apply to young people.

Our findings can only *suggest*, of course, just what the recommended daily allowances for young people should be. The amounts of ascorbic acid needed by guinea pigs and human beings to prevent scurvy are about the same when figured on a body-weight basis [about 0.2 mg/kg per day (5, 6)]. Since our results indicate that young guinea pigs need about 250 times this much, it is reasonable to conclude that human needs are probably correspondingly high.

On a body-weight basis, which must be accepted only tentatively, the need of 5.0 mg of ascorbic acid per 100 g of body weight per day in young guinea pigs for healthful development is equivalent to the need on the part of a developing 30-kg youngster for 1500 mg of ascorbic acid daily, rather than the Food and Nutrition Board's recommendation of 40 mg.

On the basis of the amount of ascorbic acid produced in the body of rats (which synthesize their own supply at the rate of about 26 mg/kg of body weight per day) (7), it would be predicted that for healthful growth guinea pigs would need about 130 times the amount needed to protect against scurvy. If the same ratio applies to human beings, this would mean a requirement of about 26 mg/kg of body weight per day or about 780 mg of ascorbic acid per day for a 30-kg youngster.

All bases of estimation lead to the conclusion that for young guinea pigs and young human beings alike, the needs of ascorbic acid for healthy development are much higher than have been accepted. It appears that for young people the need is probably at least 20 times higher than the accepted "recommended daily allowance." Individual needs vary over a wide range.

The implication of these findings goes much further. If this gross uncertainty in human requirements for ascorbic

acid prevails in nutritional science, can there not be comparable uncertainty with respect to other vitamins, such as vitamin A and vitamin D? The whole concept of "daily allowances" set at specific figures is also open to question, and in an era when nutrition has not been incorporated into medical science, vast uncertainty exists with respect to what the human needs may be for many individual nutrients—minerals, amino acids, and vitamins.

I am grateful to Lynn Old and Yvonne Lo for their expert assistance in these experiments.

1. Waugh, W. A. & King, C. G. (1932) *J. Biol. Chem.* **97**, 325–331.
2. Pauling, L. (1970) *Vitamin C and the Common Cold* (W. H. Freeman and Co.).
3. Stone, I. (1966) *Perspect. Biol. Med.* **10**, 133–134.
4. Williams, R. J. & Deason, G. (1967) *Proc. Nat. Acad. Sci. USA* **57**, 1638–1641.
5. Collins, M. & Elvehjem, C. A. (1958) *J. Nutr.* **64**, 503–511.
6. Abt, A. F., von Schuching, S. & Enns, T. (1963) *Amer. J. Clin. Nutr.* **12**, 21–29.
7. Burns, J. J., Mosbach, E. H. & Schulenberg, S. (1954), *J. Biol. Chem.* **207**, 679–687.